

REMARKS

Claims 1-43 are pending in present application. Claims 1-17 have been considered in the present application and claims 18-43 have been withdrawn from consideration as directed to a non-elected invention. Claims 2, 6, 8-10, 14 and 16 have been amended to correlate the sequence numbering to the numbering found in the sequence listing. The specification has also been amended to correlate the sequence numbering to the numbering found in the sequence listing. The amendments to the claims and specification are supported by the application as filed, do not add new matter, and are otherwise proper. Applicant respectfully requests entry of this amendment in its entirety.

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the following remarks.

Election/Restriction

In the Office Action, claim 43 was not examined "because although it recites a cis-acting element, the element is encoded by a different sequence, SEQ ID NO:3 and which represents a separate and distinct invention and further would require a serious search burden." Applicant respectfully disagrees that the search regarding SEQ ID NO:3 would constitute a serious burden on the Examiner. A review of the two sequences reveals that not only are they related, SEQ ID NO:3 includes 100 percent of the sequence information of SEQ ID NO:1. In other words, all of SEQ ID NO:1 can be found within SEQ ID NO:3. Additionally, both sequences relate to the CD11d promoter and adjoining areas. Thus, a search of SEQ ID NO:1 would automatically include a search of a substantial portion of SEQ ID NO:3. Accordingly, applicant respectfully requests the Examiner examine claim 43 in the present application.

Rejection of claims under 35 U.S.C. §112

In the Office Action, claims 1, 3-7, 11-13, 15 and 17 were "rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that

the inventor, at the time the application was filed, has possession of the claimed invention." Applicant respectfully traverses this rejection.

"There is a strong presumption that an adequate written description of the claimed invention is present in the application as filed." MPEP 2163 I.A. (emphasis added).

"Consequently, rejection of an original claim for lack of written description should be rare." MPEP 2163 II.A. (emphasis added).

In order to "satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude the inventor had possession of the claimed invention." MPEP §2163.I.

"Possession may be shown in many ways. For example, possession may be shown by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas[.]" MPEP §2163.II.3. For "biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length." 2163 II.A.3.(a).

Applicant has shown he possessed the claimed promoter by reducing the invention to practice as described in the examples. Example 1 describes the identification of the promoter, example 2 describes the isolation and sequence analysis of the CD11d 5' flanking region, example 3 describes the determination of the transcription start site of the CD11d gene, example 4 describes the functional analysis of the CD11d promoter including the identification of several sequences that include the sequence that provides the promoter activity, example 5 describes the interaction of DNA binding proteins with the CD11d sequence, example 6 describes sequence deletions that reduce activity of the promoter, example 7 regulation of the CD11d promoter region, and example 8 describes myeloid-specific downregulation of CD11d by PMA. Accordingly, it cannot be disputed that the applicant has actually reduced the claimed promoter to practice and this reduction to practice shows the applicant possessed the claimed invention.

Additionally, the applicant has demonstrated possession of the invention by providing the complete sequence information of the CD11d promoter and gene and several of their characteristics in exacting detail. Specifically, FIGS. 1 and 9 provide the complete sequence information for the CD11d promoter and the sequence information for the introns and exons of the CD11d gene as well as several putative binding sites for transcription factors on these sequences. FIG. 2 depicts a restriction map of this sequence as well as the location of the exons of the CD11d gene, and FIG. 3 shows the determination of the transcriptional start site for the CD11d gene. This clearly demonstrates that the applicant had possession of the entire sequence and also possession of all parts of the sequence.

Accordingly, applicant has described the sequence or structure of the claimed promoter as well as demonstrated the function and activity of the promoter and by doing so has clearly provided a written description and demonstrated possession of the claimed invention. Therefore, applicant respectfully requests the Examiner withdraw this rejection.

The Office Action also stated that claims 1-17 were rejected as indefinite. However, the Office Action makes no statement as to why claims 1, 3-7, 11-13, 15 and 17 are indefinite. Accordingly, applicant respectfully requests the Examiner withdraw this rejection with respect to claims 1, 3-7, 11-13, 15 and 17.

Claims 2, 8, 9, 10, 14 and 16 were rejected as "vague and indefinite" because "the means used by Applicant to designate the region of SEQ ID NO:1 is unclear because they do not relate to the number assigned to SEQ ID NO:1 in the sequence listing." Applicant has amended the sequence numbering in these claims to correspond to the numbering found in the sequence listing and respectfully request the Examiner withdraw this rejection.


Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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MARKED UP VERSION SHOWING CHANGES MADE

Below are the marked up replacement paragraph(s):

Paragraph starting page 2, line 15:

A preferred myeloid cell specific promoter is all or a functional portion of isolated or recombinant SEQ ID NO:1, such that the sequence is sufficient to direct myeloid cell specific expression of a gene. More preferably, the myeloid cell specific promoter of the present invention is the -946 to +74 region in FIG. 1 that is upstream (5') of the CD11d gene, as identified by positions 225 to 1244 of SEQ ID NO:1. In particular, the present invention relates to portions of this sequence that are sufficient to direct myeloid cell specific expression of a heterologous gene, and also includes modifications of this sequence that retain sufficient activity to direct myeloid cell specific expression of a heterologous gene. More preferably, the invention relates to the region from -173 to +74 that is upstream (5') of the CD11d gene in FIG. 1 (positions 998 to 1244 of SEQ ID NO:1). The invention further includes all or a functional portion of isolated or recombinant SEQ ID NO:1, wherein the sequence comprises one or more modifications, such that the modified sequence retains sufficient activity to direct myeloid cell specific expression of a gene.

Paragraph starting on page 3, line 10:

A preferred *cis*-acting element is the -173 to +74 region that is upstream (5') of the CD11d gene as shown in FIG. 1 (positions 998 to 1244 of SEQ ID NO:1). Also preferred is the -72 to -40 region (positions 1099 to 1131 of SEQ ID NO:1), more specifically, the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1), that is upstream (5') of the CD11d gene as shown in FIG. 1. In particular, the invention is directed to *cis*-acting elements that positively influence the CD11d promoter, increasing the activity of the promoter.

Paragraph starting on page 3, line 15:

Cis-acting elements that negatively influence the CD11d promoter are also incorporated as part of the present invention. Particularly, the present invention relates to the -591 to -378 region that is upstream (5') of the CD11d gene as shown in **FIG. 1 (positions 580 to 793 of SEQ ID NO:1)**. This *cis*-acting element contains a cell-specific silencer element.

Paragraph starting on page 5, line 5:

FIG. 1 shows the DNA sequence of the CD11d 5'flanking region and exons 1-7 and shows the sequence analysis data for the -946 to +74 CD11d promoter region (**positions 225 to 1244 of SEQ ID No: 1**). The complete sequence in lowercase for introns 1, 3 and 4, and the sequence at the intron-exon junctions for introns 2, 5, 6 and 7 and their sizes are shown. Consensus gt/ag splice junctions are underlined. Putative binding sites for transcription factors are underlined and indicated above the sequence. The transcriptional start site (+1), the ATG translational start site (boxed), and protein sequence (SEQ ID NO:2) are shown.

Paragraph starting on page 8, line 10:

It has been surprisingly and unexpectedly discovered that the -946 to +74 region (**positions 225 to 1244 of SEQ ID NO:1**), as illustrated in FIG. 1., contains the promoter activity for the CD11d gene. More specifically, it has been surprisingly and unexpectedly discovered that the -173 to +74 region (**positions 998 to 1244 of SEQ ID NO:1**) is sufficient to confer leukocyte-specific expression of a gene in myeloid cells, when the gene is a heterologous gene, such as a luciferase reporter gene.

Paragraph starting on page 9, line 18:

A preferred *cis*-acting element is the -173 to +74 region that is upstream (5') of the CD11d gene as shown in **FIG. 1 (positions 998 to 1244 of SEQ ID NO:1)**. This *cis*-acting element regulates cell-specific downregulation of CD11d by 4-phorbol-12-myristate 13-acetate (PMA). Also preferred is the -72 to -40 (**positions 1099 to 1131**

of SEQ ID NO:1), more specifically, the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1) that is upstream (5') of the CD11d gene. This *cis*-acting element binds the transcription factor Sp1. It has been found that purified Sp1 binds at the -63 to -40 (positions 1108 to 1131 of SEQ ID NO:1) region that is upstream (5') of the CD11d gene; whereas crude protein extract that contains Sp1 binds to a larger region, namely, the -72 to -40 (positions 1099 to 1131 of SEQ ID NO:1) region that is upstream (5') of the CD11d gene. It has been discovered that a functional Sp1 binding site is necessary for high levels of CD11d promoter activity. The presence of these *cis*-acting elements positively influence the CD11d promoter, increasing the activity of the promoter.

Paragraph starting on page 10, line 4:

Cis-acting elements that negatively influence the CD11d gene are also incorporated as part of the present invention. Most notably, it has been discovered that the -591 to -378 region [of] shown in FIG. 1 (positions 580 to 793 of SEQ ID NO:1) contains a cell-specific silencer element. As described in the following Examples, evidence of a cell-specific silencer element within the -591 to -378 region (positions 580 to 793 of SEQ ID NO:1) that serves to downregulate CD11d expression in Jurkat and IM-9 cells was found.

Paragraph starting on page 14, line 28:

A series of 5'-unidirectional deletions of the -946 to +74 region (positions 225 to 1244 of SEQ ID No: 1) of the CD11d promoter were prepared by the polymerase chain reaction (PCR) with oligonucleotide primers specific to this region and fused to the firefly luciferase gene (*luc*) in plasmid pGL3-Basic (Promega Corp., Madison, WI). The forward and reverse primers used in the PCR contained XhoI and HindIII restriction sites, respectively, for cloning of the final PCR product into pGL3-Basic. The -500 to +93 region of the CD11a promoter (positions 671 to 1263 of SEQ ID NO:1), the -500 to +50 region of the CD11b promoter (positions 671 to 1220 of SEQ ID NO:1), and the -196 to +30 region of the CD11c promoter (positions 975 to 1200 of SEQ ID NO:1) were prepared in a similar manner and ligated into pGL3-Basic by the methods of Noti *et al.* and

Noti. Noti, J. D., *et al.*, Mol. Cell. Biol. 16:2940-2950 (1996); Noti, J. D., J. Biol. Chem. 272:24038-24045 (1997).

Paragraph starting on page 15, line 9:

A primer containing a deletion of the Sp1-binding site (-63 to -40, positions 1108 to 1131 of SEQ ID NO:1) was used in the PCR to construct reporter plasmid CD11d(-173/+74)(-63/-40)-*luc*. The plasmid pPacSp1, which expresses Sp1 from the *Drosophila* actin promoter, and the control plasmid pPacO, containing only the *Drosophila* actin promoter, were additionally utilized. The construction of plasmids that express Sp2 and Sp3 from the actin promoter (plasmids pPacSp2 and pPacSp3, respectively) were prepared as described by Noti, J. D., J. Biol. Chem. 272:24038-24045 (1997). The integrity of all constructs was verified by DNA sequence analysis.

Paragraph starting on page 16, line 17:

The PCR was performed to prepare a double-stranded probe to the -173 to +74 region (positions 998 to 1244 of SEQ ID NO:1) and one primer was labeled with [γ^{32} P]ATP. The probe was purified by electrophoresis through a 2% agarose gel on to NA45-DEAE paper according to the manufacturer's instructions (Schleicher and Schuell, Keene, NH). Approximately $1-2 \times 10^5$ cpm of probe (1-2 ng), and either 50 μ g crude nuclear extract protein (prepared according to Noti *et al.*, DNA and Cell Biol. 11:123-138 (1992)) or 1 to 4 footprinting unit (fpu, concentration determined by the manufacturer) (Promega Corp., Madison, WI) of purified Sp1 protein, were incubated in a total volume of 50 μ l binding buffer containing 0 or 5 μ g poly d(I-C), 6.25 mM MgCl₂, 50 mM KCl, 0.5 mM EDTA, 10% glycerol, 0.5 mM DTT, and 25 mM Tris-HCl, pH 8.0, for 15 min. at room temperature. Then 50 μ l of 5 mM CaCl₂, 10 mM MgCl₂, and 0.2-2 units of DNase I were added. After 1 min. at room temperature the reaction was stopped with 90 μ l of 0.2 M NaCl, 0.03 M EDTA, 1% SDS, 10 μ g *Escherichia coli* tRNA, phenol\chloroform extracted, ethanol precipitated, and analyzed on a sequencing gel.

Paragraph starting on page 17, line 20:

5' CTGGGAGAAGGAAGCCAGGTC 3' (SEQ ID NO:9) (for first strand synthesis from the denatured DNAs), which spanned the region -171 to -151 (positions 1000 to 1020 of SEQ ID NO:1); primer #2,

Paragraph starting on page 17, line 23:

5' CAGGTTGTGGAGGGGGACAGAATGAGG 3' (SEQ ID NO:10) (amplification primer), which spanned the region -146 to -120 (positions 1025 to 1051 of SEQ ID NO:1); and primer #3,

Paragraph starting on page 17, line 25:

5' GGTTGTGGAGGGGGACAGAATGAGGGTTTTTCC 3' (SEQ ID NO:11) (labeling primer), which spanned the region -144 to -112 (positions 1027 to 1059 of SEQ ID NO:1). First strand synthesis was done for 30 min. at 60°C. The DNAs were denatured for 4 min. at 95°C and amplified by the PCR (18 cycles) as follows: 1 min. at 95°C, 2 min. at 68°C, and 3 min. at 76°C. An extra 5 seconds was added to each extension step and the final extension proceeded for 10 min. Two additional cycles of the PCR were carried out to label the PCR products as follows: 1 min. at 95°C, 2 min. at 69°C, and 10 min. at 76°C. An approximately equal amount of each sample was loaded on a sequencing gel. The band intensities were analyzed on a Storm Phosphoimager.

Paragraph starting on page 18, line 7:

The -381 to +74 region of CD11d (positions 790 to 1096 of SEQ ID NO:1) was amplified by the PCR and cloned into the XhoI and HindIII sites of pGEM-7Zf(-) (Promega Corp., Madison, WI). This clone was linearized with XhoI, and used as template in an in vitro transcription system to prepare a 562 bp RNA probe (Riboprobe, Promega Corp., Madison, WI) that spanned this region according to the manufacturer's instructions. The RNA probe was labeled with [α -32P]UTP to a specific activity of 7×10^7 cpm/ μ g, loaded on to a 5% polyacrylamide/8 M urea gel, and subsequently eluted into buffer containing 0.5 M ammonium acetate/1 mM EDTA/0.2% SDS. Approximately $1-2 \times 10^5$ cpm of

probe was annealed to either 20 µg total RNA from HL60 or yeast cells and hybridized in 20 µl of 80% deionized formamide/100 mM sodium citrate pH 6.4/300 mM sodium acetate pH 6.4/1mM EDTA for 16-18 hr. at 44°C. Following hybridization, the annealed probe/RNA complexes were treated with various concentrations of RNase A/T1 (Ambion, Inc., Austin, TX), extracted with proteinase K and phenol/chloroform, and analyzed on a 5% polyacrylamide/8 M urea gel.

Paragraph starting on page 18, line 22:

For S1 nuclease analysis, a 247 nucleotide single-stranded DNA probe corresponding to the -173 to +74 region of CD11d (positions 998 to 1244 of SEQ ID NO:1) was prepared by extension of a 22 nucleotide long primer on the luciferase reporter plasmid containing this region. The probe was end-labeled with [α -32P]ATP (probe specific activity of 5.3×10^7 cpm/µg). Hybridization of the DNA probe, prepared with the Prime-A-Probe Kit (Ambion, Inc., Austin, TX), to either 500 ng THP1 poly (A+) RNA or 20 µg yeast total RNA and subsequent digestion with S1 nuclease was performed according to the instructions in the S1-Assay Kit provided by the manufacturer (Ambion Inc, Austin, TX). A second antisense DNA probe, 99 nucleotides long with the 5'-end positioned 19 bp upstream of the ATG codon, was chemically synthesized, end-labeled with [α -32P]ATP (probe specific activity of 2.88×10^8 cpm/µg) and hybridized with HL60 poly (A+) RNA or yeast total RNA.

Paragraph starting on page 20, line 3:

As illustrated in FIG. 1, the CD11d genomic clone was found to contain exons 1-7 not previously known in the art, which correspond to nucleotides 1-704 of the CD11d coding sequence (positions 1171-1874 of SEQ ID NO:1). Exon 8 and possibly another exon is predicted to lie between nucleotides 704-982 of CD11d (positions 1874-2152 of SEQ ID NO:1), which would be consistent with an average exon size of 100-150 nucleotides.

Paragraph starting on page 21, line 29:

CD11d is expressed predominately on myeloid cells and exposure to phorbol ester led to its downregulation from the cell surface. THP1 cells were transfected with construct CD11d(-946/+74)-*luc*, which contains the -946 to +74 region of CD11d (**positions 225 to 1244 of SEQ ID NO:1**) fused to the luciferase gene, and 24 hr. post-transfection were exposed to PMA for varying times, as shown in FIG. 4. Transfected THP1 cells exposed to PMA for up to 10 hr. showed no decrease in luciferase expression, however, after 24 hr., luciferase activity decreased 55%, as shown in FIG. 4. For comparison, expression of CD11a, which is detected in all leukocytes, and CD11c, which is predominately detected in myeloid cells, were monitored following transfection of the CD11a-*luc* and CD11c-*luc* constructs, respectively. Luciferase activity from CD11a-*luc* in THP1 cells was increased 4.5-fold, and luciferase activity from CD11c-*luc* was increased 8.3-fold in the presence of PMA, as illustrated in FIG. 4. These results show that chronic, rather than acute, exposure to PMA leads to downregulation of CD11d expression (and upregulation of CD11a and CD11c expression as expected), and that one or more *cis*-acting elements within the -946 to +74 region (**positions 225 to 1244 of SEQ ID NO:1**) mediates this effect.

Paragraph starting on page 22, line 15:

The -946 to +74 region (**positions 225 to 1244 of SEQ ID NO:1**) was further examined to localize the *cis*-acting element(s) responsible for PMA-induced downregulation of CD11d and/or other elements that influence either basal or cell-specific expression. A series of CD11d reporter constructs containing progressively larger 5'-deletions was prepared and transfected into various cell lines. Luciferase expression from the constructs transfected into THP1 cells varied, but not significantly.

Paragraph starting on page 22, line 21:

CD11d(-173/+74)-*luc*, which contains only the -173 to +74 region (**positions 998 to 1244 of SEQ ID NO:1**) of CD11d, retained all of the activity obtained with CD11d(-946/+74)-*luc* and was 43-fold higher than that obtained with the promoterless pGL-3

Basic plasmid, as shown in FIG. 5A. In contrast, luciferase expression in MCF-7 breast cancer cells transfected with these constructs was increased only 2.5 to 6.4-fold over that obtained with pGL-3 Basic as shown in FIG. 5B. Expression from CD11d(-946/+74)-*luc* transfected into the B-cell line IM-9 was also reduced, as luciferase activity was only 8.1-fold over that obtained from pGL3-Basic, as illustrated in FIG. 5C. CD11d(-946/+74)-*luc* expression in the T-cell line Jurkat, was also reduced, although expression was higher than anticipated as it was 18-fold greater than that obtained from pGL3-Basic, as shown in FIG. 5D. Deletion of the -591 to -378 region (positions 580 to 793 of SEQ ID NO:1) resulted in significant increases in luciferase expression in both IM-9 and Jurkat cells to approximately the level of expression of CD11d(-173/+74)-*luc* in THP1 cells[]. See FIGS 5C and 5D, compare CD11d(-591/+74)-*luc* with CD11d(-378/+74)-*luc*. This result indicates the presence of a cell-specific silencer. Luciferase expression from each of the CD11d-*luc* 5'-deletion constructs transfected into THP1 cells was reduced to approximately the same extent after exposure to PMA, as shown in FIG. 5A. A similar response to PMA was confirmed in another myeloid cell line, HL60, wherein it was discovered that PMA reduces expression in HL60 by 77%. This shows that a PMA-responsive *cis*-acting element(s) lies within the -173 to +74 region (positions 998 to 1244 of SEQ ID NO:1), since CD11d(-173/+74)-*luc*, which contains only this region, responds to PMA. In contrast, luciferase expression in IM-9 and Jurkat cells transfected with the CD11d-*luc* 5'-deletion constructs was not reduced by PMA, but instead, was increased 1.5 to 2.5-fold, see FIGS. 5C and 5D. Together, these results show two regions of CD11d that regulate its expression. The -591 to -378 (positions 580 to 793 of SEQ ID NO:1) region may contain a cell-specific silencer element, and the -173 to +74 region (positions 998 to 1244 of SEQ ID NO:1) regulates cell-specific downregulation of CD11d by PMA[].

Paragraph starting on page 23, line 21:

DNase I footprint analysis was performed to determine whether DNA binding proteins interact with the -173 to +74 region (positions 998 to 1244 of SEQ ID NO:1). When nuclear extracts prepared from unstimulated and PMA-stimulated THP1 cells were

added to a probe labeled on the coding strand, strong protection of the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1) was revealed. This same region was also protected by nuclear extracts prepared from unstimulated and PMA-stimulated Jurkat and IM-9 cells. When a probe labeled on the non-coding strand was used, strong protection of an overlapping region, -72 to -45 (positions 1099 to 1126 of SEQ ID NO:1), was detected with unstimulated and PMA-stimulated nuclear extracts from all three cell lines. DNA sequence analysis of the overlapping region revealed the presence of an Sp1 binding site.

Paragraph starting on page 24, line 1:

In vitro DNase I footprint analysis showed that purified Sp1 protein could also protect the -63 to -40 region positions 1108 to 1131 of SEQ ID NO:1. Electrophoretic mobility shift analysis (EMSA) with THP1 nuclear extract protein and a probe to the -63 to -40 region positions 1108 to 1131 of SEQ ID NO:1 revealed a protein/DNA complex that could be supershifted with anti-Sp1 antibody.

Paragraph starting on page 24, line 7:

To determine whether the Sp1-binding site is important for CD11d expression, this site was deleted from CD11d(-173/+74)-*luc* and its effect on expression was monitored in transfected cells. Deletion of the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1) resulted in reduction of luciferase expression from CD11d(-173/+74)(-63/-40)-*luc* to 24% in transfected THP1 cells, as shown in FIG. 6A. When transfected THP1 cells were exposed to PMA, expression from CD11d(-173/+74)-*luc* was reduced to 30%. Deletion of the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1) further reduced luciferase expression from CD11d(-173/+74)(-63/-40)-*luc* in PMA-stimulated THP1 cells only an additional 7%, as shown in FIG. 6A. Luciferase expression from CD11d(-173/+74)(-63/-40)-*luc* in transfected IM-9 and Jurkat cells was similarly reduced to 20% and 24%, respectively, as illustrated by FIGS 6B and 6C. Although PMA did not reduce the expression of luciferase from CD11d(-173/+74)-*luc* transfected into IM-9 and Jurkat cells, expression from CD11d(-173/+74)(-63/-40)-*luc* was reduced to

18% and 20%, respectively, as shown in FIGS. 6B and 6C. These results show that the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1) is essential for CD11d promoter activity in both myeloid and non-myeloid cells. Further, the inability of PMA to reduce luciferase expression from CD11d(-173/+74)-*luc* in non-myeloid cells is dependent on the integrity of this region.

Paragraph starting on page 24, line 27:

To show that CD11d promoter activity is mediated through an interaction of Sp1 with the -63 to -40 region (positions 1108 to 1131 of SEQ ID NO:1), *Drosophila* cells, which are deficient in Sp-related proteins, were cotransfected with pPacSp1 along with CD11d(-173/+74)-*luc*, as shown in FIG. 7.

Paragraph starting on page 25, line 22:

The above results show that cell-specific downregulation of CD11d promoter activity is mediated through one or more *cis* elements within the -173 to +74 region (positions 998 to 1244 of SEQ ID NO:1). The inability of IM-9 and Jurkat cells to maintain CD11d expression in the presence of PMA when the Sp1-binding site was deleted indicated that Sp1 was a necessary factor involved in this response. Further, the possibility that loss of Sp1-binding was linked to downregulation of CD11d promoter activity in THP1 cells exposed to PMA was suggested when the reduction in luciferase activity from CD11d(-173/+74)-*luc* in transfected THP1 cells exposed to PMA was found to be about the same as that obtained in unstimulated THP1 cells transfected with the Sp1-deleted construct CD11d(-173/+74)(-63/-40)-*luc* (70% vs. 76% reduction, respectively, as shown in FIG. 6).

Paragraph starting on page 26, line 3:

In vivo genomic footprinting was performed to determine if selective Sp1-binding occurs on the CD11d promoter. Genomic DNA, methylated in vivo with dimethyl sulfate, was isolated from HL60, IM-9, and Jurkat cells that were either unstimulated or PMA-

stimulated. DNA was also isolated from HL60 and Jurkat cells, stripped of bound protein, and methylated in vitro as controls. Analysis of the CD11d non-coding strand, as shown in FIG. 8, revealed hyposensitive sites in unstimulated HL60 DNA at positions -38, -40, -42, -43, -50, -52, -55, -56, -58 to -61, -63, -65, and -66 (positions 16, 14, 12, 11, 4 and 2 of SEQ ID NO:6 and positions 22, 21, 19 to 16, 14, 12 and 11 of SEQ ID NO:5, respectively) which correspond to guanine nucleotides in the Sp1-binding site. No protection was seen over these positions on genomic DNA from PMA-stimulated HL60 cells. In contrast, genomic DNAs from IM-9 and Jurkat cells, either unstimulated or PMA-stimulated, were similarly protected over these positions. From these results, we conclude that occupation of the CD11d promoter by Sp1 is significantly reduced in PMA-stimulated myeloid cells, and that myeloid-specific downregulation of CD11d expression is mediated through preferential loss of Sp1 binding following PMA stimulation.

Below are the marked up amended claim(s):

2. (Amended) The promoter of the CD11d gene according to claim 1, wherein the functional portion is selected from the group consisting of 225[-946] to 1244[+74] of SEQ ID NO:1, and 998[-173] to 1244[+74] of SEQ ID NO:1.

6. (Amended) The promoter of the CD11d gene according to claim 5, wherein one of the strands of the DNA hybridizes to all or a functional portion of a sequence selected from the group consisting of 225[-946] to 1244[+74] of SEQ ID NO:1, and 998[-173] to 1244[+74] of SEQ ID NO:1.

8. (Amended) The *cis*-acting element according to claim 7, wherein the *cis*-acting element is the 225[-946] to 1244[+74] region of SEQ ID NO:1.

9. (Amended) The *cis*-acting element according to claim 7, wherein the *cis*-acting element is the 999[-173] to 1244[+74] region of SEQ ID NO:1.

10. (Amended) The *cis*-acting element according to claim 7, wherein the *cis*-acting element is the 1099[-72] to 1131[-40] region of SEQ ID NO:1.

14. (Amended) The *cis*-acting element according to claim 13, wherein the binding site for Sp1 is the 1108[-63] to 1131[-40] (5') region of SEQ ID NO:1.

16. (Amended) The *cis*-acting element according to claim 15, wherein the *cis*-acting element is the 580[-591] to 793[-378] region of SEQ ID NO:1.